dlc — 24.07.86 — 12.17.8 — dlt

88-170966/25 B03 YAMS 25.10.86 *J6 3107-924-A

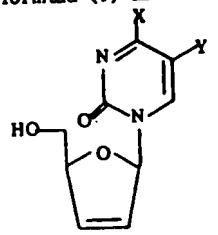
YAMASA SHOYU KK

25.10.86-JP-254552 (12.05.88) A61k-31/50 C07d-405/04

Anti:retro:virus drug used to treat aids, etc. - contg. specified 2', 3'-di:dehydro: pyrimidine nucleoside as active component

C88-076211

Antiretrovirus drug contains 2',3'-didehydropyrimidine-nucleoside of formula (I) as active component.



(I)

X = amino or OH;
Y = H when X is amino;
Y = methyl when X is OH.

B(4-B3A, 7-A1, 7-D4C, 12-A6, 12-D2A, 12-G5) 6 B0127

USE/ADVANTAGES

(I) is useful as remedy for Acquired Immune Deficiency Syndrome (AIDS) or Adult T Cell, Leukaemia (ATL). It has excellent antiretrovirus activity and high safety.

(I) can be formulated e.g. as tablets or encapsulated powders.

EXAMPLE

2', 3'-Dideoxy-3'-5'-anhydrocitydine (4.8g) was dissolved in a DMSO soln. (150 ml) contg. t-BuOK. Resultant soln. was stirred at room temp. for 2 hrs. then concentrated under reduced press. to dryness. The residue was dissolved in 20 ml of water and passed through a weak acidic cation exchange resin Amberlite IRC-50 (H^+ form) column to effect neutralisation. The eluate was concentrated to dryness and residue was dissolved in 30% methanol and developed on strong basic anion exchange resin Dowex-1 (OH-form) column (2 x 45 cm) eluating with methanol-water.

Fractions contg. end cpd. were collected, concentrated to dryness and crystallised from ethanol to give 2',3'-dideoxy-2',3'-didehydrocytidine (dd-Cyd) (2.9 g) in 61% yield, m.pt. 160 - 162°C. (6ppW69EDDwgNo0/0) J63107924-A

88-170986/25 B02 MINO- 27.10.86 *J6 3107-981-A

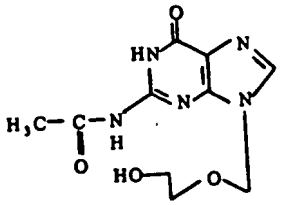
MINOFARGEN SEIYAKU

27.10.86-JP-253618 (12.05.88) A61k-31/52 C07d-473/18

New N2-acetyl derivs. of 9-((2-hydroxyethoxy methyl)-guanine - useful as therapeutic agents for infectious diseases e.g. varicella

C88-076231

9-[(2-Hydroxyethoxy)methyl]-N^2-acetylguanine of formula (I) is new:



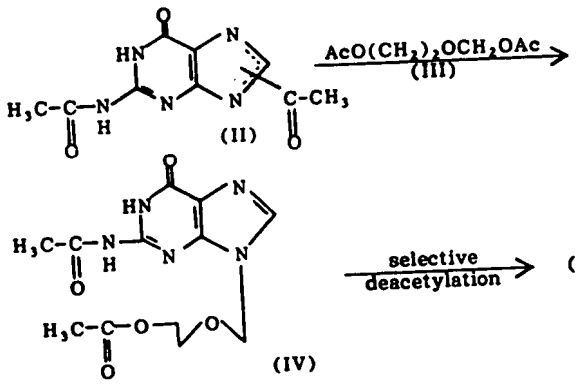
(I)

USE

(I) is an antiviral agent for therapy of infectious diseases such as varicella.

B(6-D9, 12-A6) N(5-E2) 2 B0141

PREPARATION



A catalyst (e.g. p-toluenesulphonic acid, nitrobenzene-p-sulphonic acid, aniline-p-

J63107981-A+

88-170987/25 B02 MINO- 27.10.86 *J6 3107-982-A

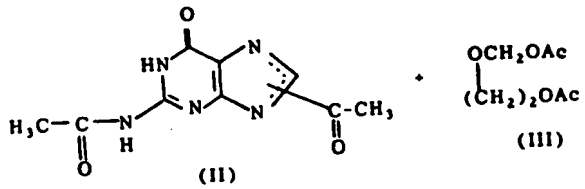
MINOFARGEN SEIYAKU

27.10.86-JP-253619 (27.10.86) C07d-473/18

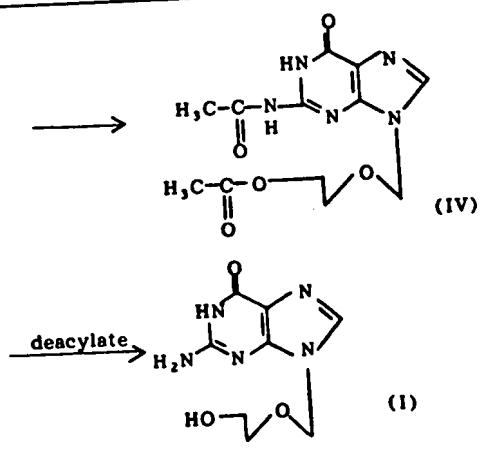
9-((2-hydroxyethoxy)methyl) guanine prepn. - by reacting di:acetyl guanine with 2-oxa-1,4 butane:diol di:acetate, then de:acetylating

C88-076232

Preparation of 9-[(2-hydroxyethoxy)methyl]-guanine (I) comprises reacting diacetylguanine (II) and 2-oxa-1,4-butanedioldiacetate (III) to obtain 9-[(2-acetoxyethoxy)-methyl]-N^2-acetylguanine (IV) then deacetylating.



B(6-D9, 12-A5, 12-A6) 2 B0142



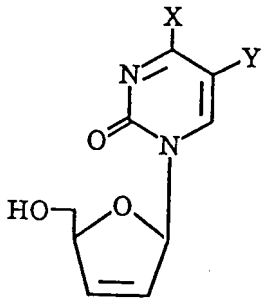
J63107982-A+

Kokai Tokkyo Koho 63-107924 (5/12/88)
Appl. No: 61-254552 (10/25/86)
Applicant: Yamasa Shoyu Co., Ltd.

Antiretroviral agent

Claim

Antiretroviral drugs containing 2',3'-didehydropyrimidine nucleosides of formula I:



[I]

wherein X represents an amino group or hydroxy group, Y represents hydrogen when X is an amino group and Y represents a methyl group when X is a hydroxy group, as an active ingredient.

Description

Technical field

This invention relates to antiretroviral drugs.

Background of the invention

Acquired Immuno Deficiency Syndrome (AIDS) and Adult T Cell Leukemia (ATL) are caused by retroviral infections and the origins of the outbreak have just recently been elucidated. It is known that in AIDS cases, the T cell of human lymphocyte is selectively infected with Human Immuno Virus which causes AIDS and then immuno deficiency is caused by cytophathosis which is the specific killing of infected T cells [Journal of Clinical and Experimental Medicine (Igaku No Ayumi), 137(12), 973 (1986)].

No effective therapy for AIDS has yet been established, however, several countermeasures are considered and three therapeutic attempts are currently being undertaken [The Pharmaceuticals Monthly (Gekkan Yakuji), 28(3), 61-65 (1986)].

A. Treatment with antiviral agents effective against HIV

Suramin, ribavirin, HPA-23, azidothymidine and interferon as antiviral agents against HIV are currently the most studied. However, there are still many problems related to the use of all of those agents due to insufficient efficacy and/or serious side effects.

B. Treatment with immuno stimulants

This treatment is a method whereby the immunological competence which has been depressed by a virus is restored to normal with immuno stimulants. Applications of IL-II, isoprinosine and other agents are being attempted for this purpose. As this treatment is just as an anosotropic treatment, it is necessary to use antiviral agents, such as those described above, together with the immuno stimulants.

C. Vaccine

In the past, many viral diseases have been exterminated or remarkably reduced through administration of a vaccine. However, the development of an AIDS vaccine is thought to be very difficult due to frequent antigenic variation of the HIV.

Problems which will be resolved by this invention

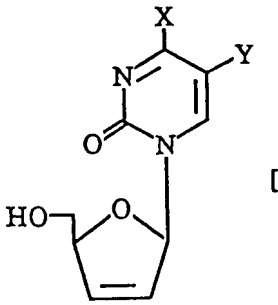
Although several compounds having anti-HIV activity have been reported as described above, the development of antiviral agents with excellent antiretroviral activity with lower toxicity, few side effects and the ability for long term continuous use is highly desired.

Therefore, it is the purpose of this invention to provide a drug with excellent antiretroviral activity and high safety.

Methods to resolve the problem

The inventors in studying novel pyrimidine nucleoside derivatives useful as antiretroviral drugs, discovered that 2',3'-didehydropyrimidine nucleosides have excellent antiretroviral activity and completed this invention.

This invention provides an antiretroviral drug containing 2',3'-didehydropyrimidine nucleoside of formula I:



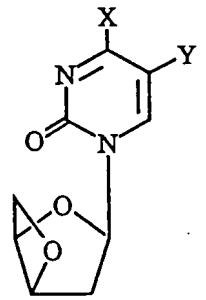
[I]

wherein X represents an amino group or hydroxy group, Y represents hydrogen when X is an amino group and Y represents a methyl group when X is a hydroxy group, as the active ingredient.

Specific examples of 2',3'-didehydropyrimidine nucleoside represented by formula I which is the active component of the drugs provided by this invention are 2',3'-dideoxy-2',3'-didehydrocytidine (dd-Cyd) or 3'-deoxy-2',3'-didehydrothymidine (dd-Thd).

The pharmaceutically acceptable acid addition salts thereof

i.e. the hydrochloric acid salt, the sulfuric acid salt and others are included. These compounds are known and synthesized by treating 2',5'-anhydropyrimidine nucleoside of formula II:



[II]

[wherein X and Y are the same as above]
with potassium t-butoxide in dimethylsulfoxide [J. Org. Chem., 31, p205 (1966) and J. Org. Chem., 32, p817 (1967)].

Dimethylacetamide, dimethylformamide, hexamethylphosphoric triamide and other aprotic polar solvents may also be used as a solvent and sodium methoxide, sodium isopropoxide, diazabicycloundecene, n-butyl lithium and other strong bases may also be applied in this reaction. The reaction is performed for a few hours at 0-100°C.

The product may be isolated by conventional methods, such as extraction, recrystallization, adsorption chromatography and ion exchange chromatography.

The drug provided by this invention are used for the treatment of retroviral infectious diseases.

Although the dosage of 2',3'-dehydropyrimidine nucleosides must be carefully adjusted, according to the physician's judgement based on the gravity of the illness and drug tolerance of the patient, the compound may be administered from 0.1 to 10 g, preferably from 0.2 to 5g per day, by one or more doses for an average adult. The dosage regimen may take other suitable forms according to the administration route.

The drug in this invention may be prepared for administration by optional common pharmaceutical methods. Therefore, this invention includes the pharmaceutical composition containing 2',3'-dehydropyrimidine nucleoside of formula I.

This composition with optional desired pharmaceutical carrier or adjuvants may be administrated by common methods.

The pharmaceutical composition for oral administration is provided in a suitable form for absorption from the digestive tract and prepared as a solid dosage i.e. tablet, capsule, powder, granule and the like and liquid dosage i.e. syrup, suspension, elixir and the like. The solid composition may be prepared with a binder i.e. syrup, arabian rubber, gelatin, sorbit, tragacanth, polyvinylpyrrolidone and the like, a vehicle i.e. lactose, sugar, cornstarch, calcium phosphate, sorbit, glycine and the like, a lubricant i.e. magnesium stearate, talc, polyethyleneglycol, silica and the like, a lysicagent i.e. potato

amylumn, humectant, stabilizer, flavor and the like as adjuvants. The liquid composition may be prepared with a suspending agent i.e. sorbit, syrup, methylcellulose, glucose/sugar syrup, gelatin, hydroxymethylcellulose, carboxymethylcellulose, aluminum stearate gel, hydrogenated edible oil and the like, an emulsifier, preservative i.e. methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, sorbic acid and the like as adjuvants.

The pharmaceutical composition for injection i.e. subcutaneous, intramuscular and intravenous injection can be prepared with 2',3'-didehydropyrimidine nucleoside, which is the active ingredient of this invention, a pH regulator, buffer, stabilizer, preservative, or solubilizer and the like in a conventional manner.

The effect of the invention

Experimental results on the anti-HIV effects of 2',3'-didehydro-pyrimidine nucleosides are shown below

Test 1
Measurement of anti-HIV activity of the drug by determination of cell proliferation rate

In this test, MT-4 cells, established human T cell line which is adult T cell leukemia virus I (HTLV-I) positive [Gann Monogr., 28, p217-228 (1982)], and HTL-III type virus, a kind of HIV, were used.

MT-4 cells ($3x10^5$ cell/ml) infected with a HTL-III type virus were inoculated into RPMI 1640 medium containing 10% of fetal bovine serum without complement, 100IU/ml of penicillin and 100mcg/ml of streptomycin with various concentrations of drugs and incubated in a CO_2 gas incubator at 37°C. Three day later, one half of each culture was removed and the number of surviving cells ($x10^4$/ml) and their survival ratio (%) were determined respectively by the trypan blue staining method. An equal amount of medium containing the same concentration of drug was added to each of the remaining cultures. After an additional three-day incubation period, the number of surviving cells were counted.

The measured values are shown in table 1. The figures on the table show the number of survival cells ($x10^4$/ml) with the survival ratio shown in parenthesis.

Table 1

drugs / incubation days / conc. of drug	dd-Thd day 3	dd-Thd day 6	dd-Cyd day 3	dd-Cyd day 6
10 (mcg/ml)	169 (92)	100 (84)	-	-
2	161 (92)	101 (87)	161 (94)	124 (78)
1	163 (95)	104 (84)	168 (91)	90 (80)
0.5	182 (94)	98 (80)	169 (95)	92 (78)
0.25	181 (93)	90 (82)	170 (93)	93 (72)
0.125	189 (94)	89 (76)	167 (84)	33 (48)
0	42 (53)	4 (7)	42 (53)	4 (7)

Table 1 indicates that the killing of cells by the HTL-III virus could be inhibited with dd-Thd (>0.125mcg/ml) and dd-Cyd (>0.25mcg/ml), whereas no growth in the control cells (0mcg/ml of drug) could be seen on day 3 and nearly all had been killed on day 6.

Test 2
The effect of the drug on T-cell growth

The effect of the drug on the growth of MT-4 cells not infected with the HTL-III virus was observed as in test 1. The results are shown in table 2. The values in the table show the numbers of surviving cells (x10^4/ml), while survival ratios are shown in parenthesis.

Table 2

drugs / incubation days / conc. of drug	dd-Thd day 3	dd-Thd day 6	dd-Cyd day 3	dd-Cyd day 6
50 (mcg/ml)	86 (66)	8 (1)	4 (8)	0 (0)
25	130 (87)	57 (66)	16 (19)	0 (0)
10	168 (94)	116 (88)	58 (70)	1 (15)
2	163 (93)	124 (93)	185 (95)	124 (93)
0	181 (93)	131 (93)	181 (93)	131 (93)

Table 2 shows that the growth of MT-4 cell was not effected by dd-Thd (<10mcg/ml) and dd-Cyd (<2mcg/ml).

Test 3
Assay of anti-HIV activity with immunofluorescence

The survival cells detected in test 1 were each dried on slides, fixed with cold methanol for 3 minutes and treated with 1/1000 dilution of anti-HIV-III active human serum for 30 minutes at 37°C. Then the slides were washed with phosphate buffer-saline (PBS) for 15 minutes and treated with fluorescein-isothiocyanate bonded anti-human IgG for 30 minutes at 37°C. Again, the slides were washed with PBS and the number of cells emitting fluorescence were measured by a fluorescent microscope.

The results are shown in table 3. The values in the table show the ratio (%) of the number of fluorescent emitting cells to

the number of whole cells.

Table 3

drugs	dd-Thd		dd-Cyd	
incubation days	day 3	day 6	day 3	day 6
conc. of drug				
10 (mcg/ml)	0	0	-	-
2	0	<1	0	<1
1	0	<1	0	<1
0.5	1	<1	1	<1
0.25	1	4	3	8
0.125	2	9	15	32
0	47	100	47	100

As shown in table 3, less than 1% of the group treated with dd-Thd or dd-Cyd (>0.5mcg/ml) were viral antigen positive and expressions of viral antigen were distinctly inhibited, but almost 100% of the control group not administered the drug were antigen active.

Test 4

Assay of anti-HIV activity via the viral growth inhibition method

MT-4 cells infected with HTLV-III were incubated for 4 days with various concentrations of the drugs as in test 1. The viral quantity discharged into the culture medium during incubation was then measured with a plaque assay as follows.

One ml of poly-L-lysin solution (50mcg/ml) was dropped onto each 35mm plastic petri dish. The petri dish was allowed to stand for 1 hour at room temperature. Thus, the petri dishes coated with poly-L-lysin were prepared. Each dish was washed three times with PBS, then MT-4 cells (1.5ml of 150×10^4cell/ml) were added and treated for 1 hour at ambient temperature. The dishes were washed gently twice with PBS, then a supernatant (100mcl) of the said culture infected with HTLV-III in the presence of the drug was added slowly and the virus was adsorbed on standing for 1 hour. 1ml of an agarose layered medium (RPMI 1640 medium containing 10% fetal bovine serum, antibiotic and 0.6% agarose) was added to each dish and incubated in a CO_2 incubator for 3 days at 37°C, then 1ml of agarose layered medium containing neutral red was added, the cultures incubated for 3 days, then the amount of virus measured.

The assay described above was repeated three times and the average amount of virus was calculated.

The results are shown in table 4. Figures in the table represent an amount of virus (PFU/ml±S.D., N=3).

Table 4

conc. of drug	drug dd-Thd	dd-Cyd
10 (mcg/ml)	<10	<10
1	1.0±1.0 x10	<10
0.1	1.2±0.31x10^4	11.0±3.0x10^4
0	25.7±2.9 x10^4	25.7±2.9x10^4

As shown in table 4, the number of virus in the groups treated with the drug decreased significantly, but that of the control group without the drug was 25.7x10^{4}PFU/ml.

Test 5
Assay of anti-HIV activity using the plaque method

A viral solution (100ml, 2000PFU/ml) was added gently to MT-4 cell cultures in each poly-L-lysine coated petri dish as prepared in test 4 above and virus was adsorbed on standing for 1 hour. 1 ml of agarose layered medium was added to each dish which was then incubated in a CO_2 incubator for 3 days at 37°C, then 1 ml of agarose layered medium containing neutral red was added. Each dish was incubated for 3 days at 37°C and plaque numbers were measured.

The assay described above was repeated three times and the average was calculated.

The results are shown in table 5. Values in table indicate the average plaque numbers (/dish ± S.D., n=3).

Table 5

conc. of drug	drug dd-Thd	dd-Cyd
5 (mcg/ml)	0	0
0.5	0	0
0.1	0	0
0.05	21.3±2.5	91±11.8
0.01	183.7±7.2	216±18.1
0	192 ±22.8	192±22.8

As shown in table 5, although the number of plaques formed on the untreated control group totaled 192, groups treated with dd-Thd or dd-Cyd (0.05mcg/ml) formed only 21.3 plaques and 91 plaques respectively and no plaque formation was observed at higher concentration levels of either drug.

As shown by the results described above, 2',3'-didehydropyrimidine nucleosides have anti-HIV activity at low concentration without effecting the host cell. Therefore this invented drug is useful as an antiretroviral agent having low toxicity.

Examples

The following provides examples of this invention. It should be noted however, that possible samples are not limited to those given below.

Example 1 Tablet

dd-Thd	10g
cornstarch	65g
carboxycellulose	20g
polyvinylpyrrolidone	3g
calcium stearate	2g
total weight	100g

According to a conventional method, 100mg tablets were prepared. Each tablet contains 10mg of dd-Thd.

Example 2 Powder, Capsule

dd-Cyd	20g
cellulose crystalline	80g
total weight	100g

Both flours are mixed to form a powder. 100mg of the powder is packed into a No.5 hard capsule to form a capsule.

Preparation 1 Synthesis of dd-Thd

Potassium t-butoxide (3.6g) was added to 100ml of dimethylsulfoxide, followed by the addition of 3.6g of 3'-deoxy-3',5'-anhydrothymidine. The mixture was then stirred for 2 hours at room temperature. The reaction mixture was neutralized by an acetic acid-ethanol solution and evaporated in vacuo at 50°C. The residue was suspended to acetone and insoluble salts was filtrated then the filtrate evaporated. The residue was dissolved in 50ml of ethanol to which 250ml of benzene was added. The solution was then concentrated to 50 ml to yield 3.2g of crystal. It was recrystallized from ethanol-benzene and 2.9g of dd-Thd (80% yield) was obtained.

Mp: 165-166°C

UV absorption: $\lambda_{max}^{H_2O}$ 266nm (ϵ 9910)

Anal.

calc'd for $C_{10}H_{12}N_2O_4$:
C, 53.56 H, 5.40 N, 12.50

found: C, 53.39 H, 5.43 N, 12.38

Preparation 2 Synthesis of dd-Cyd

To a solution of potassium t-butoxide (5.3g) in 150ml of dimethylsulfoxide was added 4.8g of 2',3'-dideoxy-3',5'-anhydrocytidine and the mixture was stirred for 2 hours at room temperature. The reaction mixture was evaporated in vacuo at about 50°C, then the residue was dissolved in 20ml of water and the solution neutralized by being passed through a column of Amberlite IRC-50 (H^+ type). The eluent was evaporated and the residue dissolved in 50 ml of 50% methanol. The solution was

then chromatographed on Dowex-1 (OH^- type) and eluted with methanol-water. The fractions containing the desired compound were combined and evaporated. The residue was crystallized from ethanol to obtain 2.9g of dd-Cyd (61% yield).

Mp: 160-162°C

UV absorption: λ_{max}^{H2O} 271nm (ε 8710)

Anal.

calc'd for $C_9H_{11}N_3O_3$:

C, 51.67 H, 5.30 N, 20.09

found: C, 51.89 H, 5.41 N, 19.94